

REMARKS

Claims 1-15 are pending in the present application.

Entry of the above amendments is earnestly solicited.
An early and favorable first action on the merits is earnestly requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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Attachments

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

The claims have been amended as follows:

3. Use according to Claim ~~1~~ or ~~Claim 2~~,
characterized in that the nucleotide sequences coding for a
protein of the TMAO reductase system are chosen from those of
the following bacteria:

- marine bacteria, such as those of the genus *Shewanella*,
Photobacterium or *Vibrio*, the said sequences being chosen in
particular from the following:

* the sequence coding for the TorA protein of
Shewanella massilia shown in Figure 1,

* the sequence coding for the TorA protein of
Shewanella putrefaciens shown in Figure 1,

* the sequence coding for the TorA protein of
Shewanella c shown in Figure 2,

* the partial sequence coding for the TorA protein of
Photobacterium phosphoreum shown in Figure 3,

* the sequence coding for the TorC protein of
Shewanella massilia shown in Figure 14,

- bacteria obtained from brackish water, such as those of
the genus *Rhodobacter*, or *Roseobacter*, the said sequences
being chosen in particular from the following:

* the sequence coding for the DorA protein of *Rhodobacter sphaeroides* shown in Figure 4,

* the sequence coding for the DorA protein of *Rhodobacter capsulatus* shown in Figure 4,

* the sequence coding for the DorC protein of *Rhodobacter sphaeroides* shown in Figure 14,

- enterobacteria, such as those of the genus *Escherichia*, or *Salmonella*, the said sequences being chosen in particular from the following:

* the sequence coding for the TorA protein of *Escherichia coli* shown in Figure 4,

* the partial sequence coding for the TorA protein of *Salmonella typhimurium* shown in Figure 5,

* the sequence coding for the TorC protein of *Escherichia coli* shown in Figure 14.

4. Use according to ~~any one of the Claims 1 to 3~~, Claim 1, characterized in that the nucleotide sequences are used in the form of pairs of primers chosen from any one of the following three groups of primers:

(1) the group of primers "DDN" comprising:

♦ the following compositions of nucleotide sequences "DDN+":

- DDN1+ : 5' CGG vGA yTA CTC bAC hGG TGC 3' : mixture of 54 nucleotide sequences,

- DDN5+ : 5' ATy GAT GCG ATy CTC GAA CC 3' : mixture of 4 nucleotide sequences,

♦ the following compositions of nucleotide sequences "DDN-":

- DDN2- : 5' CGT Amw sGT CGA kAT CGT TrC GCT C 3' : mixture of 32 nucleotide sequences,

- DDN3- : 5' GAC TCA CAy Awy TGy GAG TG 3' : mixture of 16 nucleotide sequences,

- DDN4- : 5' TGr CCd CGr kCG TTA AAG AC 3' : mixture of 24 nucleotide sequences,

- DDN5- : 5' CCv GGT TCG AGr ATC GCA TC 3' : mixture of 6 nucleotide sequences,

(2) the group of primers "BN" comprising:

♦ the following compositions of nucleotide sequences "BN+":

- BN1+ : 5' C bGA yAT CsT rCT GCC 3' : mixture of 16 nucleotide sequences,

- BN3+ : 5' GGm GAY TAY TCb ACm GGy GC 3' : mixture of 96 nucleotide sequences,

- BN6+ : 5' Twy GAR CGy AAC GAY mTC GA 3' : mixture of 64 nucleotide sequences,

♦ the following compositions of nucleotide sequences "BN-":

- BN2- : 5' GG vyC rTA CCA bsC vCC TTC 3' : mixture of 216 nucleotide sequences,

- BN4- : 5' ATC Arr CCn swv GGC GTG CC 3' : mixture of 192 nucleotide sequences,

- BN5- : 5' GbC ACr TCd GTy TGy GG 3' : mixture of 72 nucleotide sequences,

(3) the group of primers "BC" comprising:

♦ the following compositions of nucleotide sequences "BC+":

- BC1+ : 5' ACn CCn GAr AAr TTy GAr GC 3' : mixture of 256 nucleotide sequences,

- BC2+ : 5' TGy ATy GAr TGy CAy AAr GG 3' : mixture of 96 nucleotide sequences,

♦ the following compositions of nucleotide sequences "BC-":

- BC2- : 5' CCy TTr TGr CAr TCd ATr CA 3' : mixture of 96 nucleotide sequences,

- BC3- : 5' TTn GCr TCr AAr TGn GC 3' : mixture of 128 nucleotide sequences,

in which $n = (A, C, G, T)$, $y = (C, T)$, $r = (A, G)$, $h = (A, C, T)$, $d = (G, A, T)$, $m = (A, C)$, $w = (A, T)$, $b = (G, T, C)$, $s = (G, C)$, $v = (G, A, C)$, and $k = (G, T)$,

the pairs of primers being chosen in such a way that one of the primers of a pair corresponds to one of the aforementioned compositions of nucleotide sequences DDN+, BN+ or BC+, whereas the other primer corresponds respectively to one of the aforementioned compositions of nucleotide

sequences DDN-, BN- or BC-, the said pairs of primers being chosen in particular from any one of the following four pairs:

(a) the pair DDN1+/DDN5-, leading to amplification of fragments of the gene coding for the TorA protein in bacteria, of a size of about 820 base pairs (bp), and especially to the amplification of an 821 bp fragment of the gene coding for the TorA protein in bacteria of the genus *Shewanella*, such as the 821 bp fragment bounded by the nucleotides located in positions 620 to 1450 of the *torA* gene of *S. massilia* shown in Figure 4,

(b) the pair BN6+/BN2-, leading to amplification of fragments of the gene coding for the TorA protein in bacteria, with a size of about 710 bp, and especially to the amplification of a 727 bp fragment of the gene coding for the TorA protein in bacteria of the genus *Shewanella*, such as the 727 bp fragment bounded by the nucleotides located in positions 1657 to 2403 of the *torA* gene of *S. massilia* shown in Figure 4,

(c) the pair BN6+/BN4-, leading to amplification of fragments of the gene coding for the TorA protein in bacteria, with a size of about 360 bp, and especially to the amplification of a 355 bp fragment of the gene coding for the TorA protein in bacteria of the genus *Shewanella*, such as the 355 bp fragment bounded by the nucleotides located in

positions 1657 to 2023 of the *torA* gene of *S. massilia* shown in Figure 4,

(d) the pair BC1+/BC2-, leading to amplification of fragments of the gene coding for the TorC protein in bacteria, with a size of about 170 bp, and especially to the amplification of a 197 bp fragment of the gene coding for the TorC protein in bacteria of the genus *Shewanella*, such as the 197 bp fragment coding for the polypeptide fragment bounded by the amino acids located in positions 114 to 179 of the TorC protein of *S. massilia* shown in Figure 14.

5. Use according to ~~one of the Claims 1 to 4~~, Claim 1, characterized in that the hosts that can be carriers of bacteria involved in the process of degradation of the flesh of aquatic animals, as described in Claim 3, are aquatic organisms, especially marine organisms such as fish and crustacea, and more particularly Atlantic fish such as sole, cod, or fish from the Mediterranean Sea such as surmullet and sea bream, as well as certain animals from fresh or brackish water.

6. Use according to ~~any one of the Claims 1 to 5~~, Claim 1, for implementing a method of detecting the presence of all bacteria involved in the degradation of the flesh of aquatic animals, within the framework of a method of evaluating the freshness of aquatic animals from which the sample tested was

taken, when the said animals are removed from their natural environment.

10. A method of detecting all bacteria involved in the degradation of the flesh of aquatic animals in a host that can be a carrier of the said bacteria, the said method being effected starting from a biological sample taken from the said host, the said biological sample corresponding in particular to a subcutaneous fragment of flesh of the aquatic animal in question, and being characterized in that it comprises a step of hybridization of at least one nucleotide sequence as defined in ~~any one of the Claims 1 to 9,~~ Claim 1, with fragments of genes coding for a protein of the TMAO-reductase system of bacteria involved in the degradation of the flesh of aquatic animals that can be present in the biological sample taken from the said host, followed by a step of detection, in particular by electrophoresis, of the possible presence, in the said sample, of genes coding for a protein of the TMAO-reductase system, or of fragments of these genes, of which the number of copies has been amplified if necessary.

13. A kit for implementing a method of detection ~~according to one of the Claims 10 to 12,~~ characterized in that it comprises:

- one or more nucleotide sequences or primers defined in ~~one of the Claims 1 to 9,~~ Claim 1,

- a DNA polymerase,
 - a reaction medium advantageously consisting of 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01% gelatin, the 4 constituent deoxynucleotides of the DNAs (dCTP, dATP, dGTP, dTTP) at a concentration of 100 μ M each.
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